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SYNTHESIS AND CYTOTOXIC ACTIVITY OF WRIGHTIADIONE AND ITS DERIVATIVES

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Dedicated to Prof. Dr. Somsak Ruchirawat on the occasion of his 80th anniversary

Abstract – A synthetic method for generating the tetracyclic isoflavone wrightiadione was developed through a Friedel-Crafts acylation of isoflavone-2-carboxylic acid. A series of wrightiadione derivatives was conveniently synthesized by this approach and evaluated for cancer cytotoxic, cancer chemopreventive, and antimalarial properties. Some derivatives exhibited potent cancer cytotoxic activity at the single-digit micromolar level.

Wrightiadione is a unique tetracyclic isoflavone ring system in which the D-ring contains a ketone moiety. Isolation of wrightiadione and its structural determination were first reported in 1992 from *Wrightia tomentosa*,[1] and later from *Wrightia pubescens*[2] and *Wrightia religiosa*.[3] This natural product was also reported to have cytotoxic activity against leukemia[1] and HT29 cell lines.[4] However, in late 2018, the structural elucidation of wrightiadione was reinvestigated, which led to the conclusion that the previously known natural product wrightiadione was a misidentification of the isobaric (same nominal mass) and isosteric (same number of atoms, valency, and shape) natural product tryptanthrin.[5] Therefore, wrightiadione can no longer be said to be a natural product and those previous bioactivity data of the isolated natural product were indeed belonged to tryptanthrin (**Figure 1**).

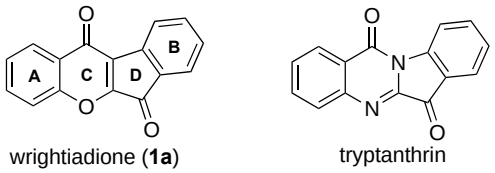
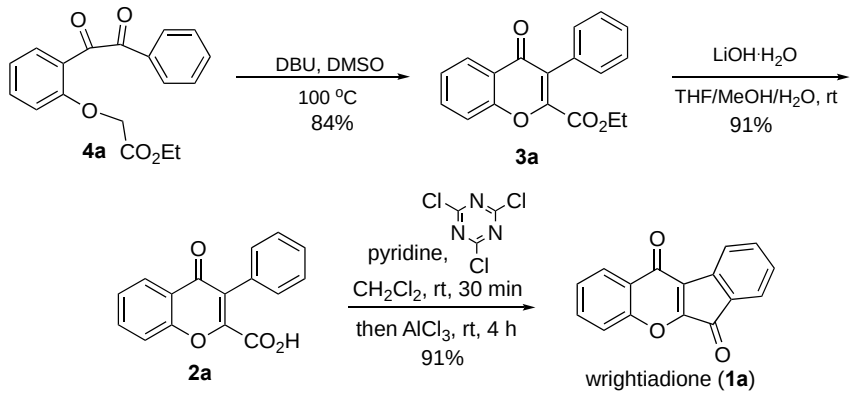


Figure 1. Structure of wrightiadione and tryptanthrin

To date, four total syntheses of the corrected wrightiadione structure (**1a**) have been published,[6-9] and in fact the first reported total synthesis by Ruchirawat and Thasana acknowledged some discrepancies between the NMR data of the synthesized compound and the isolated compound from *W. tomentosa*,[6] which considered as the first clue for this misidentification of the isolated compound. For the biological activity of the corrected wrightiadione structure (**1a**), thus far only one study has been reported, whereby the docking simulation studies and the enzymatic kinase assays revealed a remarkable selectivity of wrightiadione (**1a**) toward TrKa and PLK3 compared to other cancer-related kinases.[10]

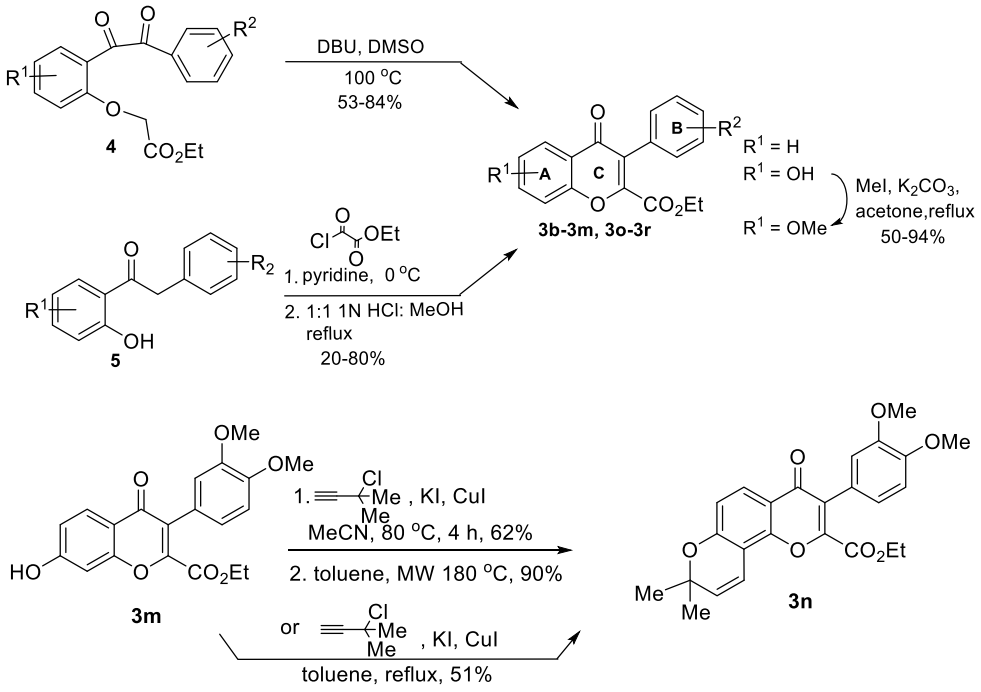
Motivated by the abovementioned finding regarding the inhibitory activity and limited information of other biological properties of the corrected wrightiadione structure, we were interested in developing a method for synthesizing wrightiadione (**1a**) and its derivatives, and investigating their biological activities. Accordingly, the present work describes another total synthesis of wrightiadione and its derivatives using Friedel-Crafts acylation of isoflavone-2-carboxylic acid, and evaluation of cancer cytotoxicity, cancer chemoprevention, and antimalarial activities of these compounds.

Based on the premise that wrightiadione is indeed an isoflavone with an additional five-membered ketone moeity, we consequently developed a synthetic strategy for wrightiadione **1a** by elaboration of the isoflavone structure. Our synthetic plan was to construct the ketone D-ring of wrightiadione via a Friedel-Crafts acylation reaction from isoflavone-2-carboxylic acid **2a**. Thus, the aryl diketone phenoxy ether **4a**, which was prepared in three steps from iodophenol, underwent base induced cyclization using DBU in DMSO at 100 ˚C to give ethyl isoflavone-2-carboxylate **3a** in 84% yield (Scheme 1).[11] The ethyl ester moiety was subsequently hydrolyzed using $LiOH{\cdot}H_2O$ to give the required isoflavone-2-carboxylic acid **2a** in 91% yield.[12] We next proceeded to the formation of ketone moiety to complete the teracyclic ring system of the wrightiadione structure. Various conditions of the Friedel-Crafts acylation reaction were screened with compound **2a**, and it was found that the Friedel-Crafts acylation conditions using cyanuric chloride and $AlCl_3$[13] at room temperature gave excellent yield for D-ring formation and generated wrightiadione (**1a**) in 91% yield in one pot process.



Scheme 1. The total synthesis of wrightiadione

To expand the biological profiles of wrightiadione derivatives by focusing on the introduction of substituents on ring A and ring B (see Table 1), several ethyl isoflavone-2-carboxylate **3b**–**r** were then prepared following the previously established protocols[12] as depicted in Scheme 2. The ethyl isoflavone-2-carboxylate **3b-c**, which have no substituent on ring A, were prepared by base induced cyclization of the aryl diketone phenoxy ethers **4**. The ethyl isoflavone-2-carboxylate **3d**–**m** and **3o-r**, possessing mono- or dioxygenated group on ring A, were obtained by cyclization of their corresponding deoxybenzoins **5** with ethyl chlorooxoacetate[12,14] and the respective methoxy derivatives were then synthesized by methylation reaction. For compound **3n**, the chromene ring was prepared by the copper catalyzed etherification with chloro-3-methylbut-1-yne under refluxing toluene in which the reaction readily proceeded to cyclization to **3n** in 51% yield.[12,15]

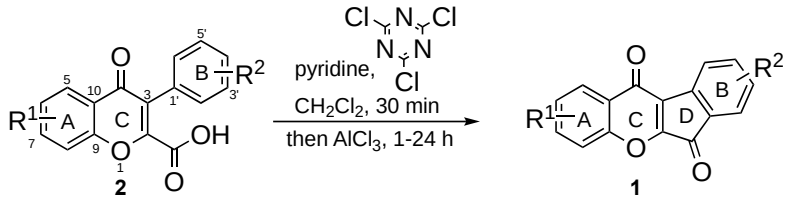


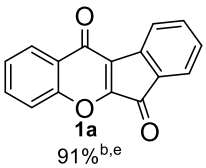
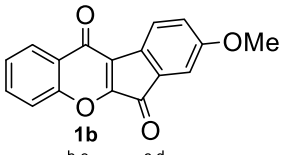
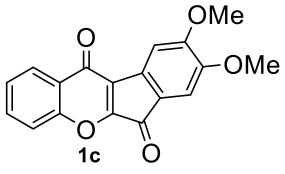
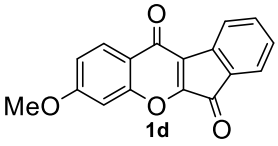
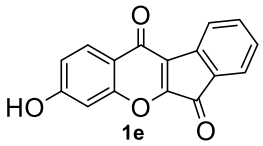
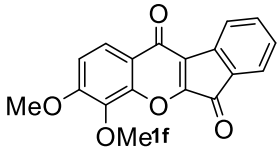
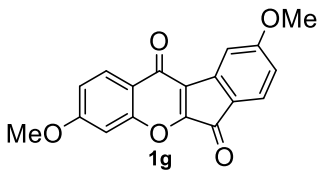
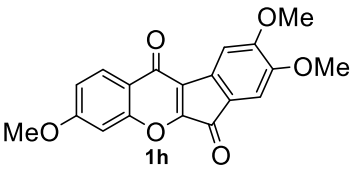
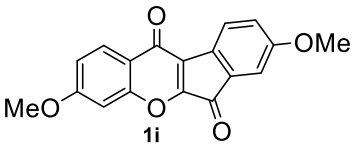
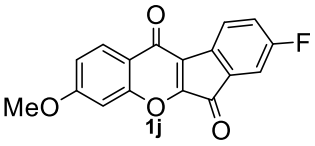
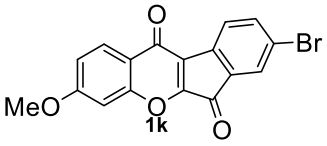
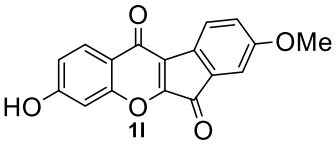
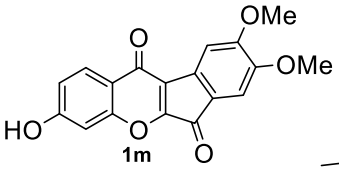
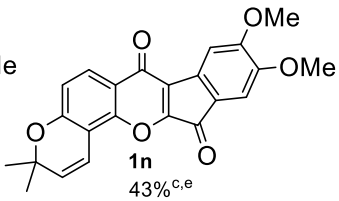
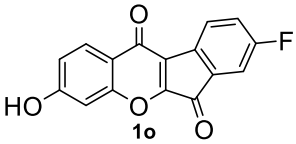
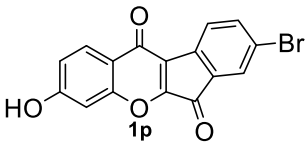
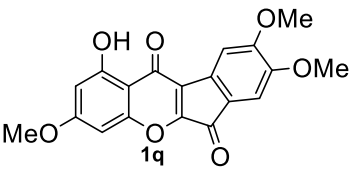
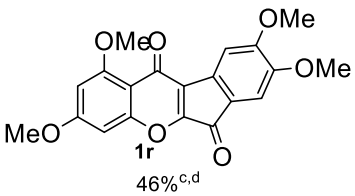
Scheme 2. Preparation of the ethyl isoflavone-2-carboxylate

After the ester moiety in **3** was hydrolyzed to give the corresponding isoflavone-2-carboxylic acids **2**,[12] the compounds were then subjected to the Friedel-Crafts acylation reaction to generate the wrightiadione derivatives **1**. It was found that the reaction proceeded to give the wrightiadone derivatives (**1a**–**r**) in low to good yield (Table 1). Under this condition, compounds **1a**, **1d**, and **1g** were produced in high yields at room temperature. The methoxy group on the A-ring seemed to slightly affect the product yield (**1a** vs **1d**), but the hydroxy group drastically lower the yields of the products (**1d** vs **1e**), which could be observed in all other compounds containing the hydroxy group (**1l**, **1m**, **1o**, **1p**). The substituents on the arene B-ring exerted more influence on the ketone formation. When the methoxy group was placed at C-4′, the reaction required higher temperature and the product yield was much lower (**1a** vs **1b**), and this could be due to the

electron withdrawing property by inductive effect toward the reacting *meta* C-2′ position. On the other hand, the additional methoxy group at C-5′ promoted the better yield of the product (**1c**) which could be the effect of electron donating property by resonance effect toward the reacting *para* C-2′ position. The

Table 1. Synthesis of wrightiadione derivatives by Friedel-Crafts acylation reaction[a]



 **1a** 91%[b,e]	 **1b** 0%[b,e], 26%[c,d]	 **1c** 40%[b,d], 53%[c,e]
 **1d** 84%[b,d]	 **1e** 30%[c,e]	 **1f** 45%[b,e]
 **1g** 90%[b,e]	 **1h** 77%[c,d]	 **1i** 39%[c,e]
 **1j** 74%[c,f]	 **1k** 68%[c,d]	 **1l** 36%[c,e]
 **1m** 29%[c,e]	 **1n** 43%[c,e]	 **1o** 23%[c,f]
 **1p** 26%[c,e]	 **1q** 37%[c,d]	 **1r** 46%[c,d]

[a] Compound **2** (25 mg), pyridine (1 equiv.), cyanuric acid (1.6 equiv.), $AlCl_3$ (6 equiv.); [b] Reaction at room temperature; [c] Reaction at 40 °C; Reaction time after adding $AlCl_3$: [d] 1 h; [e] 4 h; [f] 24 h.

similar effects were also observed in compounds **1g**, **1h**, and **1i**. Compounds **1j** and **1k** containing halogen groups were produced in good yields.

Cytotoxic activities were assessed on five cancer cell lines including human hepatocarcinoma (HepG2), acute lymphoblastic leukemia (MOLT-3), human lung cancer (A549), human cholangiocarcinoma (HuCCA-1), and acute promyelocytic leukemia (HL-60), as well as normal embryonic lung (MRC-5) cells using the 18 synthesized compounds (**1a**−**1s**). The IC_{50} values were determined after 48 h of exposure to the testing compounds to the cancer cells by using the standard MTT and XTT methods as previously described.[16] Etoposide and doxorubicin were used as the positive controls. The results are summarized in Table 2.

Table 2. Cytotoxicity (IC_{50}) of wrightiadione and its derivatives **1a-1s** against cancer cell lines[a]

Entry	compounds	HepG2[b]	MOLT-3[c]	A549[b]	HuCCA-1[c]	HL-60[c]	MRC-5[b]
1	**1a**	68.28 ± 3.53	I	I	64.37 ± 10.05	I	-
2	**1b**	21.24 ± 3.10	10.64 ± 1.44	I	15.56 ± 4.22	12.90 ± 0.57	-
3	**1c**	I	I	I	18.56 ± 0.43	I	-
4	**1d**	44.42 ± 1.83	I	I	34.97 ± 1.34	I	-
5	**1e**	111.38 ± 6.69	37.69 ± 8.59	I	129.73 ± 2.35	47.31 ± 8.10	-
6	**1f**	70.10 ± 3.00	I	I	82.64 ± 2.71	I	-
7	**1g**[d]	-	-	-	-	-	-
8	**1h**[d]	-	-	-	-	-	-
9	**1i**	24.20 ± 0.78	I	I	19.72 ± 4.98	I	-
10	**1j**	38.92 ± 5.01	14.78 ± 3.44	I	21.74 ± 1.62	12.38 ± 3.71	-
11	**1k**	5.08 ±1.15	10.89 ± 0.81	27.19 ± 1.15	18.28 ± 1.09	4.23 ± 0.08	I
12	**1l**	139.33 ± 4.78	-	I	129.82 ± 5.28	-	-
13	**1m**	19.18 ± 2.53	8.48 ± 3.27	35.10 ± 2.16	24.64 ± 5.55	9.74 ± 2.87	-
14	**1n**[d]	-	-	-	-	-	-
15	**1o**	88.37 ± 4.36	6.41 ± 1.88	125.53 ± 9.44	83.16 ± 15.48	-	138.40 ± 1.91
16	**1p**	7.99 ± 1.01	5.39 ± 0.90	91.22 ± 4.80	12.56 ± 0.68	5.95 ± 1.14	102.50 ± 5.07
17	**1q**[d]	-	-	-	-	-	-
18	**1r**	15.88 ± 0.90	I	I	14.61 ± 1.48	I	-
19	etoposide	-	0.049 ± 0.015	-	-	1.53 ± 0.25	-
20	doxorubicin	0.57 ± 0.036	-	0.19 ± 0.002	0.38 ± 0.036	-	1.88 ± 0.45

[a] The inhibitory concentration of a compound in μM necessary to achieve 50% growth inhibition (IC_{50}). Results obtained are mean values from three replicate experiments; - : not determined. I: in active (less than 50% cytotoxicity at maximum solubility value). [b] MTT assay. [c] XTT assay. [d] The compound had very low solubility in DMSO.

The parent compound wrightiadione **1a** gave only mild cytotoxic activity against the HepG2 and HuCCA-1 cell lines with IC_{50} values of 68.28 ± 3.53 μM and 64.37 ± 10.05 μM, respectively, and was inactive against MOLT-3, A549, and HuCCA-1. In each cancer cell line, several derivatives showed a significant

improvement in inhibitory activity compared to the parent wrightiadione **1a**. However, the cytotoxicity of derivatives **1g**, **1h**, **1n**, and **1q** could not be measured because of their very low solubility in DMSO.

When the B-ring of wrightiadione was substituted with a methoxy group at the C-4′ position (**1b**), the cytotoxicity appeared to be more potent than the parent compound **1a** with all cancer cell lines. However, when substituted with 4′,5′-dimethoxy substituent (**1c**), the activity only increased in the HuCCA-1 cell line, but was decreased or unaffected in the other cell lines. When the A-ring of wrightiadione was substituted, it was found that the introduction of the a methoxy group at the C-7 position (**1d**) appeared to enhance the cytotoxic activity toward HepG2 and HuCCA-1, while the introduction of a hydroxy group at C-7 (**1e**) enhanced the cytotoxic activity toward MOLT-3 and HL-60. With a methoxy substituent at C-7 on ring A, installing substituents at the C-4′ position appeared to increase the cytotoxic activity (**1i**, **1j**, **1k**) compared to the unsubstituted derivative **1d**, and among these derivatives, the bromo compound **1k** gave the highest cytotoxicity with all cancer cell lines. For the compounds with the hydroxy substituent at C-7 on ring A (**1e**, **1l**, **1m**, **1o**,**1p**), the 4′-bromo derivative **1p** gave the best cytotoxicity in most cancer cell lines except for A549, whereby the 4′,5′-dimethoxy derivative **1m** gave the best activity. Additionally, the 4′-fluoro compound **1o** also showed potent cytotoxic activity against the MOLT-3 cell line. It can also be seen that by changing the halogen substituent from fluorine to a bulkier bromine group, the cytotoxic activity towards all cell lines was obviously increased in both 7-methoxy and 7-hydroxy derivatives (**1p** vs **1o** and **1k** vs **1j**).

When considering the activity toward each cancer cell line, the wrightiadione derivatives were the least effective toward A549 cells, as only four compounds (**1k**, **1l**, **1o**, **1p**) showed mild cytotoxic activity with the best IC_{50} values only at 27.19 ± 1.15 μM from the bromo derivative **1k**. For the HuCCA-1 cell line, even though there more compounds exhibited cytotoxic activity, all of them showed only moderate to mild activity, with the best IC_{50} value only at 12.56 ± 0.68 μM from the bromo derivative **1p**. The wrightiadione derivatives seemed to exhibit good activity with HepG2, MOLT-3, and HL-60 and many exhibited an IC_{50} value at the single digit micromolar level. This included compounds **1k** and **1p** with IC_{50} values of 5.08 ± 1.15 μM and 7.99 ± 1.01 μM against HepG2, compounds **1m**, **1o**, **1p** with IC_{50} values of 8.48 ± 3.27 μM, 6.41 ± 1.88 μM, and 5.39 ± 0.90 μM against MOLT-3, and compounds **1k**, **1m**, **1p** with IC_{50}values of 4.23 ± 0.08 μM, 9.74 ± 5.95 μM, and 5.95 ± 1.14 μM against HL-60, respectively.

Over all, among all tested compounds, the bromo derivatives **1k** and **1p** showed the most potent cytotoxic activities. The bromo compound **1k** exhibited the best cytotoxic activity against HepG2, A549, and HL-60, while the bromo compound **1p** exhibited the best cytotoxic activity against MOLT-3 and HuCCA-1. Gratifyingly, these bromo derivatives **1k** and **1p** presented the high selectivity toward cancer cell lines. The bromo derivative **1k** had selectivity index (SI) with HepG2 and HL-60 of more than 5.5 and 6.6, respectively, while the bromo derivative **1p** provided a selectivity index (SI) with MOLT-3 and HL-60 of

19.0 and 17.2, respectively. It should also note that the fluoro compound **1o** exhibited good activity with MOLT-3 with a high SI value (21.6). Compound **1m** was also quite attractive as it showed strong inhibitory activity with some cancer cell lines. However, caution should be taken as the 4´,5´-dimethoxy substituent on ring B in wrightiadione derivatives is likely attributable to their limited solubility; since, in this compound series, several of the 4´,5´-dimethoxy derivatives (**1h**, **1n**, **1q**) had very low solubility in DMSO. Apart from cancer cytotoxicity, all wrightiadione derivatives were also evaluated for their cancer chemopreventive activity including radical scavenging, antioxidant, anti-inflammatory, and aromatase inhibitory activities, and some selected compounds were also tested for antimalarial properties against *Plasmodium falciparum* (strain 94). The results showed that all of the tested wrightiadione derivatives were inactive (see Table S1 and S2, Supporting information).

In summary, we present here an approach to the total synthesis of wrightiadione through a Friedel-Crafts acylation reaction of an isoflavone-2-carboxylic acid. Moreover, eighteen derivatives were synthesized and evaluated their biological activity in terms of cancer cytotoxic, cancer chemopreventive, and antimalarial properties. Even though the compounds showed no cancer chemopreventive or antimalarial properties, most derivatives displayed much higher cytotoxicity than the parent compound **1a** in several cancer cell lines. In particular, the bromo derivatives **1k** and **1p**, which exhibited the best cytotoxic activity against all tested cancer cell lines, and had low toxicity against normal cells. Therefore, these derivatives could be used as a potential lead compounds for further development as anticancer agents. Up to now, little was known about the pharmacological properties of the corrected wrightiadione structure, so this work serves as a good starting point for further biological investigations. Additionally, it is also interesting to point out that since wrightiadione and tryptanthrin have a very similar structure, they may possess similar biological activities and specific targets. Tryptanthrin is recognized for having a strong antitubercular activity which represents a potential lead for new tuberculosis (TB) drugs.[17,18] Hence, the evaluation of wrightiadione and its derivatives for the antitubercular activity should also be worthwhile.

EXPERIMENTAL

Commercial grade reagents and solvents were used as received from the supplier except where indicated otherwise. CH_2Cl_2 was dried by refluxing over CaH_2 under Argon (Ar) atmosphere. The reactions were conducted under Ar atmosphere in 13 x 100 mm screw top tubes, which were heat-dried under vacuum prior used. Thin layer chromatography was performed on Merck precoated silica gel 60 F254 plates. Silica gel 60 (Silicycle, 230–400 mesh) was used for flash column chromatography. ^{1}H- and ^{13}C-NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 using a Bruker AVANCE 400 NMR spectrometer. ^{1}H-NMR and ^{13}C-NMR chemical shifts (δ) were reported in units of part per million (ppm), relative to tetramethylsilane (TMS) as internal standard. Coupling constants (*J*) were reported in Hertz (Hz). IR spectra were recorded

on a PerkinElmer Spectrum One Spectrophotometer using a universal attenuated total reflectance (ATR) technique. HRESIMS analyses were determined using a Bruker Daltonics MicroTOF$_{LC}$ mass spectrometer.

General procedure for the synthesis of wrightiadione derivatives 1. To a solution containing 1.0 equiv. of the acid **2** in CH_2Cl_2 (0.1 M) at room temperature under Ar atmosphere, was added 1.6 equiv. of cyanuric chloride followed by 1.0 equiv. of pyridine. The solution was stirred at specified temperature for 30 min, after which time 6.0 equiv. of $AlCl_3$ was added in one portion. The mixture was further stirred for 1−24 h (until completion) and quenched with a saturated aqueous NH_4Cl solution. After stirring for additional 15 min, the mixture was extracted with a 1:4 acetone:CH_2Cl_2 solution. The combined organic layer was then washed with a saturated aqueous NaCl solution, dried with Na_2SO_4 (s), and concentrated under reduced pressure. The crude residue was purified by flash silica gel column chromatography eluted with 80% CH_2Cl_2/hexane to CH_2Cl_2 to give a wrigthiadione derivatives.

Wrightiadione (1a): Orange solid; mp 244−245 ºC; IR (neat) 3749, 1732, 1648, 1616, 1483, 1460, 1374, 1288, 1201, and 1020 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.27 (t, 1H, *J* = 7.3 Hz), 7.49 (t, 2H, *J* = 7.6 Hz), 7.59 (d, 1H, *J* = 7.3 Hz), 7.65 (d, 1H, *J* = 8.3 Hz), 7.72-7.78 (m, 1H), 7.98 (d, 1H, *J* = 7.3 Hz), and 8.31 (dd, 1H, *J* = 8.0 and 1.6 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 188.4, 175.0, 156.0, 155.9, 140.3, 136.0, 134.6, 128.7, 127.1, 127.0, 126.34, 126.27, 124.8, 123.9, and 119.4; HRMS Calcd for $[(C_{16}H_8O_3)+H]^+$: 249.0546 Found: 249.0542.

8-Methoxyindeno[2,1-*b*]chromene-6,11-dione (1b): Dark purple solid; mp 255–256 ºC; IR (neat) 3095, 2840, 1731, 1646, 1610, 1478, 1455, 1371, 1286, 1202, and 1004 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 3.85 (s, 3H), 6.94 (dd, 1H, *J* = 8.0 and 2.0 Hz), 7.18 (d, 1H, *J* = 2.0 Hz), 4.49 (t, 1H, *J* = 7.8 Hz), 7.63 (d, 1H, *J* = 7.8 Hz), 7.74 (t, 1H, *J* = 7.8 Hz), 7.86 (d, 1H, *J* = 8.0 Hz), 8.30 (d, 1H, *J* = 7.8 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 188.5, 175.0, 160.5, 155.9, 155.4, 134.4, 131.9, 128.7, 128.1, 126.3, 126.19, 126.16, 124.9, 119.4, 118.8, 112.4, and 55.8; HRMS Calcd for $[(C_{17}H_{10}O_4)+H]^+$: 279.0635. Found: 279.0663.

8,9-Dimethoxyindeno[2,1-*b*]chromene-6,11-dione (1c): Dark brown solid; mp 266–267 ºC; IR (neat) 2923, 2852, 1730, 1647, 1498, 1460, 1352, 1200, 1221, and 1055 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 3.90 (s, 3H), 4.05 (s, 3H), 7.15 (s, 1H), 7.49 (ddd, 1H, *J* = 8.6, 7.0, and 1.0 Hz), 7.57 (s, 1H), 7.64 (dd, 1H, *J* = 8.6 and 1.0 z), 7.73 (ddd, 1H, *J* = 8.6, 7.0, and 1.6 Hz), 8.28 (dd, 1H, *J* = 8.0 and 1.6 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 187.2, 174.5, 156.8, 156.0, 155.1, 148.7, 136.6, 134.3, 126.4, 126.3, 126.1, 125.5, 119.4, 118.9, 108.7, 108.1, 56.7, and 56.4; HRMS Calcd for $[(C_{18}H_{12}O_5)+H]^+$: 309.0757. Found: 309.0756.

3-Methoxyindeno[2,1-*b*]chromene-6,11-dione (1d): Orange solid; mp 242–244 ºC; IR (neat) 2948, 2840, 1732, 1637, 1618, 1602, 1475, 1436, 1242, 1176, 1102, and 1031 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 3.93, (s, 3H), 7.03 (brd, 1H, *J* = 2.3 Hz), 7.04 (dd, 1H, *J* = 8.6 and 2.3 Hz), 7.26 (t, 1H, *J* = 7.3 Hz), 7.49 (t, 1H, *J* = 7.3 Hz), 7.56 (d, 1H, *J* = 7.3 Hz), 7.96 (d, 1H, *J* = 7.3 Hz), and 8.20 (d, 1H, *J* = 8.6 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 188.4, 174.4, 164.7, 157.8, 155.7, 140.3, 135.9, 128.6, 127.51, 127.45, 127.0, 124.7, 123.9,

120.3, 115.7, 101.4, and 56.0; HRMS Calcd for $[(C_{17}H_{10}O_4)+H]^+$: 279.0652. Found: 279.0651.

3-Hydroxyindeno[2,1-*b*]chromene-6,11-dione (1e): Orange solid; mp 292–294 ºC; IR (neat) 3215, 2959, 2925, 2854, 1733, 1629, 1582, 1457, 1371, 1260, 1223, 1103, and 1029 cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 6.97- 7.01 (m, 2H), 7.31 (dt, 1H, *J* = 7.6 and 1.0 Hz), 7.51-7.57 (m, 2H), 7.79 (brd, 1H, *J* = 6.9 Hz), 7.98 (d, 1H, *J* = 9.2 Hz), and 11.08 (brs, 1H); ^{13}C-NMR (100 MHz, DMSO-d_6) δ 187.8, 173.7, 163.5, 157.4, 156.1, 140.1, 135.8, 128.6, 127.3, 127.0, 125.7, 124.5, 122.8, 118.6, 116.2, and 103.5; HRMS Calcd for $[(C_{16}H_8O_4)+H]^+$: 265.0495. Found: 265.0491.

3,4-Dimethoxyindeno[2,1-*b*]chromene-6,11-dione (1f): Brown-orange solid; mp 218–220 ºC; IR (neat) 2925, 2851, 1733, 1647, 1617, 1560, 1457, 1293, 1269, 1188, 1088, 1074, and 1042 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 4.02 (s, 3H), 4.04 (s, 3H), 7.11 (d, 1H, *J* = 9.0 Hz), 7.27 (td, 1H, *J* = 7.3 and 1.0 Hz), 7.49 (td, 1H, *J* = 7.3 and 1.2 Hz), 7.59 (brd, 1H, *J* = 7.3 Hz), 7.97 (brd, 1H, *J* = 7.3 Hz), and 8.03 (d, 1H, *J* = 9.0 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 188.1, 174.3, 157.3, 155.9, 150.4, 140.1, 137.9, 135.8, 128.6, 127.0, 126.8, 124.7, 123.8, 121.4, 121.0, 110.9, 61.9, and 56.6; HRMS Calcd for $[(C_{18}H_{12}O_5)+Na]^+$: 331.0581. Found: 331.0577.

3,9-Dimethoxyindeno[2,1-*b*]chromene-6,11-dione (1g): Orange solid; mp 299–303 ºC; IR (neat) 3093, 2848, 1719, 1643, 1620, 1601, 1472, 1434, 1346, 1295, 1240, 1177, 1112, and 1008 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 3.93 (s, 3H), 3.94 (s, 3H), 6.65 (dd, 1H, *J* = 8.2 and 2.3 Hz), 7.05 (s, 1H), 7.06 (dd, 1H, *J* = 7.3 and 2.4 Hz), 7.55 (d, 1H, *J* = 8.2 Hz), 7.58 (d, 1H, *J* = 2.3 Hz), and 8.20 (brd, 1H, *J* = 9.5 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 186.4, 174.2, 166.3, 164.6, 157.8, 157.4, 143.5, 127.4, 127.2, 125.4, 120.3, 119.6, 115.7, 111.7 (x2), 101.5, 56.01, and 56.0; HRMS Calcd for $[(C_{18}H_{12}O_5)+H]^+$: 309.0757. Found: 309.0764.

3,8,9-Trimethoxyindeno[2,1-*b*]chromene-6,11-dione (1h): Brown solid; mp 274–276 ºC; IR (neat) 2925, 2840, 1723, 1652, 1630, 1494, 1438, 1307, 1246, 1217, 1094, 1053, and 1038 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 3.90 (s, 3H), 3.92 (s, 3H), 4.04 (s, 3H), 7.00-7.06 (m, 2H), 7.14 (s, 1H), 7.56 (s, 1H), and 8.17 (dd, 1H, *J* = 8.8 and 0.3 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 187.3, 174.0, 164.5, 157.8, 156.4, 155.0, 148.6, 136.5, 127.3, 125.9, 120.3, 118.8, 115.7, 108.7, 108.1, 101.4, 56.7, 56.4, and 56.0; HRMS Calcd for $[(C_{19}H_{14}O_6)+H]^+$: 339.0863. Found: 339.0864.

3,8-Dimethoxyindeno[2,1-*b*]chromene-6,11-dione (1i): Brown solid; mp 248–252 ºC; IR (neat) 2947, 2842, 1732, 1649, 1623, 1488, 1437, 1287, 1245, 1159, 1098, and 1025 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 3.84 (s, 3H), 3.92 (s, 3H), 6.92 (dd, 1H, *J* = 8.0 and 2.4 Hz), 7.01 (d, 1H, *J* = 2.4 Hz), 7.04 (dd, 1H, *J* = 8.8 and 2.4 Hz), 7.15 (d, 1H, *J* = 2.4 Hz), 7.83 (d, 1H, *J* = 8.0 Hz), 7.18 (d, 1H, *J* = 8.8 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 188.5, 174.4, 164.6, 160.4, 157.8, 155.0, 133.8, 128.8, 128.5, 127.4, 124.8, 120.3, 118.6, 115.6, 112.4, 101.3, 56.0, and 55.8; HRMS Calcd for $[(C_{18}H_{12}O_5)+H]^+$: 309.0757. Found: 309.0758.

8-Fluoro-3-methoxyindeno[2,1-*b*]chromene-6,11-dione (1j): Orange solid; mp 246–248 ºC; IR (neat)

3070, 2961, 2929, 1735, 1644, 1621, 1483, 1438, 1347, 1255, 1156, 1102, and 1019 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 3.93 (s, 3H), 7.02 (d, 1H, *J* = 2.4 Hz), 7.05 (dd, 1H, *J* = 8.9 and 2.4 Hz), 7.15 (ddd, 1H, *J* = 8.9, 8.0, and 2.5 Hz), 7.28 (dd, 1H, *J* = 7.0 and 2.5 Hz), 7.94 (dd, 1H, *J* = 8.0 and 4.6 Hz), and 8.19 (d, 1H, *J* = 8.9 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 187.2, (*J* = 2.1 Hz), 174.2, 164.8, 163.0 (*J* = 248 Hz), 157.8, 153.3 (*J* = 3.8 Hz), 135.7 (*J* = 3.8 Hz), 128.9 (*J* = 7.3 Hz), 127.49 (*J* = 7.3 Hz), 127.46, 125.1 (*J* = 7.4 Hz), 121.1 (*J* = 22.2 Hz), 120.2, 115.8, 113.0 (*J* = 24.9 Hz), 101.3, 56.0; HRMS Calcd for $[(C_{17}H_9FO_4)+H]^+$: 297.0558. Found: 297.0553.

8-Bromo-3-methoxyindeno[2,1-*b*]chromene-6,11-dione (1k): Orange solid; mp 249–251 ºC; IR (neat) 3090, 2926, 2650, 1737, 1645, 1623, 1621, 1475, 1473, 1393, 1351, 1243, 1172, 1096, and 1027 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 3.93 (s, 1H), 7.02 (d, 1H, *J* = 2.3 Hz), 7.06 (dd, 1H, *J* = 8.9 and 2.3 Hz), 7.61 (dd, 1H, *J* = 7.8 and 1.9 Hz), 7.67 (d, 1H, *J* = 1.9 Hz), 7.85 (d, H, *J* = 7.8 Hz), 8.18 (d, 1H, *J* = 8.9 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 187.1, 174.1, 164.8, 157.8, 155.0, 138.7, 138.0, 128.5, 127.9, 127.5, 127.3, 125.1, 122.2, 120.1, 115.9, 101.3, and 56.0; HRMS Calcd for $[(C_{17}H_9BrO_4)+H]^+$: 356.9757. Found: 356.9749.

3-Hydroxy-8-methoxyindeno[2,1-*b*]chromene-6,11-dione (1l): Deep green solid; mp 384–385 ºC; IR (neat) 2956, 2924, 2853, 1723, 1622, 1466, 1288, 1259, 1096, and 1032 cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 3.80 (s, 3H), 6.97 (s, 1H), 6.99 (dd, 1H, *J* = 8.7 and 2.2 Hz), 7.05 (dd, 1H, *J* = 8.1 and 2.5 Hz), 7.14 (d, 1H, *J* = 2.5 Hz), 7.68 (d, 1H, *J* = 8.1 Hz), and 7.98 (brd, 1H, *J* = 9.1 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 187.6, 173.5, 163.5, 159.8, 157.3, 155.2, 131.6, 128.8, 127.1, 126.6, 123.8, 118.44, 118.39, 116.1, 112.0, 103.4, and 55.8; HRMS Calcd for $[(C_{17}H_{10}O_5)+H]^+$: 295.0601. Found: 295.0603.

3-Hydroxy-8,9-dimethoxyindeno[2,1-*b*]chromene-6,11-dione (1m): Brown solid; mp 346–348 ºC; IR (neat) 2966, 2927, 1724, 1652, 1587, 1499, 1471, 1298, 1217, 1133, 1062, and 1021 cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 3.78 (s, 3H), 3.89 (s, 3H), 6.94-7.01 (m, 2H), 7.14 (s, 1H), 7.36 (s, 1H), and 7.94 (d, 1H, *J* = 8.6 Hz); ^{13}C-NMR (100 MHz, DMSO-d_6) δ 186.5, 172.9, 163.1, 157.2, 156.2, 154.3, 148.1, 135.8, 127.0, 124.2, 118.5, 118.4, 116.0, 109.0, 107.2, 103.4, 56.1, and 56.0; HRMS Calcd for $[(C_{18}H_{12}O_6)+H]^+$:325.0707. Found: 325.0698.

9,10-Dimethoxy-3,3-dimethyl-3*H*-indeno[2,1-*b*]pyrano[2,3-*h*]chromene-7,12-dione (1n): Brown solid; mp 313–315 ºC; IR (neat) 2966, 2927, 1724, 1652, 1587, 1499, 1471, 1298, 1217, 1133, 1062, and 1021 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.50 (s, 6H), 3.89 (s, 3H), 4.02 (s, 3H), 5.75 (d, 1H, *J* = 10.0 Hz), 6.89 (d, 1H, *J* = 8.7 Hz), 6.92 (d, 1H, *J* = 10.0 Hz), 7.12 (s, 1H), 7.54 (s, 1H), and 8.00 (d, 1H, *J* = 8.7 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 187.1, 174.0, 157.9, 156.1, 154.8, 152.1, 148.4, 136.3, 130.8, 126.1, 125.5, 120.2, 118.8, 116.1, 114.8, 110.5, 108.6, 108.0, 78.1, 56.7, 56.3, and 28.2 (x2); HRMS Calcd for $[(C_{23}H_{18}FO_6)+H]^+$: 391.1176. Found: 391.1177.

8-Fluoro-3-hydroxyindeno[2,1-*b*]chromene-6,11-dione (1o): Brown-orange solid; mp 287–289 ºC; IR

(neat) 2922, 2326, 1734, 1625, 1586, 1480, 1369, 1249, 1109, and 1028 cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 6.98-7.05 (m, 2H), 7.38 (ddd, 1H, *J* = 9.5, 8.1, and 2.5 Hz), 7.48 (dd, 1H, *J* = 7.5 and 2.5 Hz), 7.81 (dd, 1H, *J* = 8.1 and 4.8 Hz), and 8.00 (d, 1H, *J* = 9.5 Hz); ^{13}C-NMR (100 MHz, DMSO-d_6) δ 186.4, 173.5, 163.5, 162.2 (*J* = 247 Hz), 157.3, 156.0 (*J* = 3.8 Hz), 135.9 (*J* = 3.1 Hz), 129.4 (*J* = 7.5 Hz), 127.2, 125.4, 124.0 (*J* = 7.9 Hz), 120.8 (*J* = 22.5 Hz), 118.5, 116.1, 112.7 (*J* = 25.2 Hz), and 103.4; HRMS Calcd for $[(C_{16}H_7FO_4)+H]^+$: 283.0401. Found: 283.0399.

8-Bromo-3-hydroxyindeno[2,1-*b*]chromene-6,11-dione (1p): orange solid; mp 302–305 ºC; IR (neat) 2959, 2926, 1740, 1622, 1498, 1464, 1248, 1171, and 1025 cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 7.01 (dd, 1H, *J* = 7.5 and 2.2 Hz), 7.02 (s, 1H), 7.70 (d, 1H, *J* = 1.8 Hz), 7.72 (d, 1H, *J* = 7.8 Hz), 7.77 (dd, 1H, *J* = 7.8 and 1.8 Hz), and 7.99 (brd, 1H, *J* = 9.4 Hz); ^{13}C-NMR (100 MHz, DMSO-d_6) δ 186.3, 173.4, 163.5, 157.3, 155.8, 138.9, 137.6, 129.1, 127.2, 126.9, 125.2, 124.3, 121.0, 118.4, 116.2, and 103.5; HRMS Calcd for $[(C_{16}H_7BrO_4)+H]^+$: 342.9600. Found: 342.9600.

1-Hydroxy-3,8,9-trimethoxyindeno[2,1-*b*]chromene-6,11-dione (1q): Brown solid; mp 270–274 ºC; IR (neat) 2959, 2923, 2853, 1717, 1652, 1611, 1587, 1495, 1466, 1394, 1355, 1310, 1292, 1428, 1217, 1159, 1074, 1047, and 1032 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 3.88 (s, 3H), 3.90 (s, 3H), 4.05 (s, 3H), 6.42 (d, 1H, *J* = 2.4 Hz), 6.57 (d, 1H, *J* = 2.4 Hz), 7.15 (s, 1H), 7.46 (s, 1H), and 12.44 (s, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 186.2, 178.8, 165.8, 162.6, 157.6, 157.1, 155.2, 148.6, 135.7, 124.9, 118.7, 108.8, 108.1, 107.8, 99.4, 94.0, 56.7, 56.3, and 55.9; HRMS Calcd for $[(C_{19}H_{14}O_7)+H]^+$: 355.0812. Found: 355.0807.

1,3,8,9-Tetramethoxyindeno[2,1-*b*]chromene-6,11-dione (1r): Deep purple solid; mp 235–237 ºC; IR (neat) 2925, 2853, 1723, 1649, 1619, 1586, 1498, 1461, 1352, 1302, 1215, 1162, 1093, 1066, and 1038 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 3.86 (s, 3H), 3.87 (s, 3H), 3.95 (s, 3H), 3.97 (s, 3H), 6.39 (d, 1H, *J* = 2.1 Hz), 6.59 (d, 1H, *J* = 2.2 Hz), 7.08 (s, 1H), and 7.61 (s, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 187.3, 173.8, 164.3, 161.4, 159.8, 154.7, 154.3, 148.3, 136.4, 126.7, 118.7, 111.7, 108.5, 108.1, 97.0, 93.7, 56.5, 56.31, 56.27, and 55.8; HRMS Calcd for $[(C_{20}H_{16}O_7)+H]^+$: 369.0969. Found: 369.0971.

SUPPORTING INFORMATION

Copies of the 1D- and 2D-NMR spectrum of wrightiadione (**1a**) and the ^{1}H- and ^{13}C-NMR spectrum of all synthesized wrightiadione derivatives (**1b**–**r**) are available in the Supporting Information at URL: https://www.heterocycles.jp/newlibrary/downloads/PDFsi/27610/105/1.

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